REMARKS

Claims 1 and 3-29 were pending in the above-identified application. Claims 4-7, 10-12, 16, and 24-29 have previously been withdrawn from further consideration as being drawn to a non-elected invention. Claims 1, 3, 8, 9, 13-15, and 17-23 have been acted upon by the Examiner. In light of the remarks and arguments set forth below, Applicants respectfully request reconsideration of the application. Claims 1, 19 and 20 have been amended and claim 3 has been canceled without prejudice to prosecuting the subject matter of the claim in a related co-pending application. No new matter has been added by any amendment to the claims. Applicants request reconsideration of the pending claims in view of the above amendments and the remarks below.

Rejections Under 35 U.S.C. § 102

Claims 1, 13, 14, 17, and 18 stand rejection under 35 U.S.C. § 102(b) as being anticipated by Matera *et al.* (*supra*). In particular, the Examiner continues to alleges that Matera *et al.* teaches a method of differentiating dendritic cells comprising providing a population of peripheral blood monocytes that have been selected by magnetic sorting and contacting said monocytes with GM-CSF in the absence of additional cytokines, citing to pages 30 and 31. In addition, the Examiner continues to allege that Matera *et al.* also teach culturing in a serum free medium and that the dendritic cells generated by culture with GM-CSF express increased CD1a and decreased CD14. Still further, the Examiner continues to allege that the instant claims are drawn to a method of differentiating dendritic cells employing a dendritic cell precursor, for example, a method of using a product made by a particular process, and therefore, the method by which the monocytic precursor is produced does not carry patentable weight in the absence of a structural difference. The Examiner continues to believe that the monocytic dendritic cell precursors of Matera *et al.* are the same as those produced by tangential flow filtration and furthermore, that the culture conditions comprising culture with GM-CSF taught by Matera *et al.* can be considered non-activating sine they do not result in the progress of a fully mature dendritic cell.

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Applicants must again disagree with the rejection of claims 1, 13, 14, 17 and 18 as being anticipated by Matera *et al.* In particular, although Matera *et al.* state that in some experiments cultures were started with a purified population of CD14 positive cells selected with magnetic microbeads, Applicants can find no results specifically associated with these cells. The results summarized on page 31 and depicted in the figures are explicitly stated to be from cultures of adherent monocytes induced by contact with various cytokines and with or without prolactin. As the authors did not distinguish between the results obtained with positively selected CD14 positive cells and monocytes obtained by adherence to plastic one of skill in the art must conclude that both populations of monocytes were activated. Evidence of activation is found in, for example, at page 32, right column, lines 3 and 4 wherein the authors found that GM-CSF alone was "very effective at inducing macrophage-like cells". Further, the authors found that GM-CSF and IL-4 strongly enhanced CD1a expression as compared with GM-CSF alone and CD40 was not found to be induced by GM-CSF alone. As such, the methods of Matera *et al.* do not produce dendritic cell precursors with the same characteristics. As the dendritic cell precursors obtained by Matera *et al.* are structurally different from those used in the methods recited in claims 1, 13, 14, 17 and 18, the claims are not anticipated by the reference.

Applicants respectfully request that the Examiner reconsider and withdraw the rejection of claims 1, 13, 14, 17 and 18 as being anticipated by Matera *et al.* in view of the above remarks.

Claims 1, 14, 17 and 18 remain rejected under 35 U.S.C. § 102(b) as being anticipated by Kasinrerk *et al.*, *J. Immunol.* 150:579-584, 1993. In particular, the Examiner continues to allege that Kasinrerk *et al.* teach a method of differentiating monocytes comprising providing a population of peripheral blood monocytes that have been selected by density centrifugation and negative selection, and contacting the monocytes with GM-CSF in the absence of additional cytokines. In addition, the Examiner continues to allege that Kasinrerk *et al.* teach that the resulting cell have increased expression of CD1a and decreased expression of CD14. Thus, the Examiner has concluded that Kasinrerk *et al.* describe a cell identical to that of the instant claims (*i.e.*, the cell are immature dendritic cells). Furthermore, the Examiner has concluded that the

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culture of monocytes with GM-CSF alone in the absence of additional cytokines, as taught by Kasinrerk *et al.*, can be considered non-activating conditions.

Applicants again must disagree with the Examiner's rejection of claims 1, 14, 17 and 18 as being anticipated by Kasinrerk *et al.* In particular, the Examiner has alleged that the population of peripheral blood monocytes obtained by Kasinrerk *et al.* are non-activated dendritic precursor cells because they have been isolated by density gradient centrifugation and because the isolated monocytes were shown to express CD1a when contacted with GM-CSF alone. The monocytes obtained by Kasinrerk *et al.* were not obtained by the same method as described in the present application. In particular, the method comprised the steps of density gradient centrifugation over Ficoll-Hypaque, enrichment of monocytes by collecting the E-fractions from PBMC contacted with nueraminidase-treated sheep-red blood cells, and negative selection using CD19 and CD56 monoclonal antibodies and sheep anti-mouse IgG-coated Dynabeads. the not only demonstrated an up-regulation in the expression of CD1a, but also CD1b and CD1c. These molecules can be found on both macrophage and immature dendritic cells. In addition, after further study of the cited reference, Kasinrerk *et al.* does teach that the cells obtained by the disclosed method express some CD14 as determined by Western blot (See Figure 2, lanes 1 and 4), although the level of CD14 on the cell surface was reduced as compared with the level initially found prior to treatment. As such, the method of Kasinrerk *et al.* are not the same as the claimed immature dendritic cells of the instant application. In order to more distinctly claim the method of the present application, Applicants have amended claims 1, 19 and 20 to recite "no CD14". Support for this amendment can be found, for example, in the Examples and through out the specification wherein the immature dendritic cells are described as being $CD14^-$ and Figure 1 wherein about 4% of cells after 4 days in culture were found to have CD14 on the their surface. Using standard differentiation methods for immature dendritic cells with GM-CSF alone resulted in about 21% of cells retaining CD14 on their surface. As such, Kasinrerk *et al.* does not anticipate the method of the pending claims.

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Applicants respectfully request that the Examiner reconsider and withdraw the rejection of claims 1, 14, 17 and 18 as being anticipated by Kasinrerk *et al.* in view of the above amendments and remarks.

Rejections Under 35 U.S.C. § 112

Claims 1, 3, 8, 9, 13 - 15, and 17 - 23 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The Examiner alleges the claims contain subject matter which was not described in the specification. In particular, the Examiner does not believe that the specification provides sufficient guidance to differentiate immature dendritic cells having CD1a and decreased expression of CD14 from non-activated monocytic dendritic cell precursors as broadly claims.

The Examiner alleges that the instant claims are drawn to a method of differentiating monocytic precursors into immature dendritic cells having decreased expression of CD14 and increased expression of CD1a comprising contacting non-activated monocytic precursors with GM-CSF in the absence of additional cytokines. It is the belief of the Examiner that the state of the art is such that obtaining immature dendritic cells with GM-CSF in the absence of additional cytokines is extremely unpredictable. Several references, including Chaperot *et al.*, Bernard *et al.*, Sallusto *et al.*, Matera *et al.*, and Kasinrerk *et al.*, have been cited by the Examiner as demonstrating the state of the art. Chaperot *et al.* is alleged by the Examiner to teach a method identical to that of the instant claims, including culturing the monocyte precursors in non-adherent bags, but failing to obtain $CD1a^+$ immature dendritic cells after culture in GM-CSF in the absence of additional cytokines. Chaperot *et al.* is also alleged by the Examiner to teach isolating the monocytic precursors by various methods including cytapheresis, density gradient preparation, and negative selection. Bernard *et al.* is alleged by the Examiner to also teach a method identical to that of the instant claims, including culturing in PFTE bags, but as with

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Chaperot the Examiner alleges that they fail to obtain immature dendritic cells with reduced CD14 expression by culture with GM-CSF in the absence of additional cytokines. In addition, Sallusto *et al.* is alleged by the Examiner to culture monocytic precursors in the presence of a medium containing 10% serum along with GM-CSF alone in a method disclosed in the instant specification which the Examiner alleges to prevent tight adherence and activation of the cells. The other references cited by the Examiner, Matera *et al.* and Kasinrerk *et al.*, are alleged to obtain a population of CD1a+ cells displaying decreased expression of CD14 by culture in GM-CSF in the absence of additional cytokines. As such, the Examiner does not believe that it is readily apparent which factors are critical for successfully obtaining the cells compared to the methods of Sallusto *et al.*, Bernard *et al.*, and Chaperot *et al.*

Contrary to the position of the Examiner none of the cited references teach the same method as that disclosed in the instant application or the same method as recited in the pending claims. Sallusto *et al.* teach of method of isolating monocytic dendritic cell precursors by adhesion of the monocytes to plastic. This method is well known to the skilled artisan to active the monocytes and as such, Sallusto *et al.* taught that IL-4 is required to prevent the activation and differentiation of the precursors to form macrophage. Bernard *et al.*, like Sallusto *et al.*, uses a method known to result in the production of macrophage in Teflon bags. The mononuclear cells are initially isolated by a process intended to collect platelets comprising cytapharesis and use of a Cobe 2997 continuous cell separator. The concentrated leukocyte residue was collected and resuspended in phosphate buffered saline without calcium and magnesium. Mononuclear cells were then isolated by density gradient centrifugation and countercurrent centrifugation using TS 745 solution. Subsequent to isolation the mononuclear cells were cultured for 5 to 7 days in Teflon bags in IMDM medium supplemented with L-glutamine, streptomycin, penicillin, non-essential amino acids, sodium pyruvate, 2-mercaptoethanol, human pooled AB serum, indomethacin and GM-CSF. The method to produce dendritic cells taught by Bernard *et al.* comprised culture in RPMI 1640 supplemented with L-glutamine, fetal calf serum, penicillin, streptomycin, GM-CSF and IL-4. As such, it would appear that the method that induces the

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production of macrophage either begin with activated monocytic precursor cells or the monocytes adhere to the Teflon bag during the culture period. The complete method disclosed by Bernard *et al.* is not the same as a method taught in the instant specification. As such, the skilled artisan using the specification as filed would be able to alter the method in order to obtain immature dendritic cells and not macrophage. Similarly, the skilled artisan would be able to alter the method taught by Chaperot *et al.* and obtain immature dendritic cells instead of macrophage.

Applicants have fully described and/or the prior art provides sufficient guidance to the skilled artisan to practice the method of the pending claims as required by 35 U.S.C. § 112, first paragraph. Many methods are known in the art to determine whether monocytes have been activated during isolation. In addition, methods are known or are described in the specification as filed to reduce the adhesion of non-activated monocytic dendritic cell precursors to a culture vessel. Still further, it is known from the specification that some PFTE bags used in methods for inducing the production of dendritic cells can activate monocytic dendritic cells precursors and the addition of, for example, high concentrations of an animal protein, are required to prevent the activation the monocytic dendritic cell precursors and differentiation of the cells into immature dendritic cells when cultured with GM-CSF without additional cytokines. See example 1.

Applicants respectfully request that the Examiner reconsider and withdraw the rejection of claims 1, 3, 8, 9, 13-15, and 17-23 under 35 U.S.C. § 112, first paragraph, in view of the amendments and remarks above.

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CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested. If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 206-695-1786.

Respectfully submitted,

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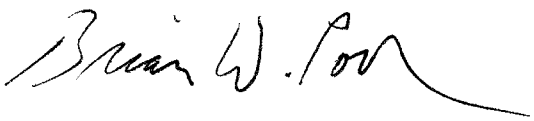

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